Gene EXPRESSion Platform: Enabling Early Potency Development and Stability Assessment

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ABSTRACT

Measuring the potency of your product throughout development is critical for program success. While assays that measure the biological activity of your product are the gold standard for potency assay measurements, these assays typically have long lead times, necessitating an alternative strategy to support early development. We have established a platform gene expression assay to bridge this gap. This poster will walk through the experiments necessary to build a robust gene expression platform assay. The result is a platform assay with a short development timeline for new products (<1 month) that can be used in the early phases of product development. This platform assay can be used to support initial assessments, including candidate screening, scale up comparability, and formulation studies. This poster will also highlight data from a stability indicating assessment that demonstrates that the gene expression platform assay is the most stability indicating assay in our toolbox, demonstrating a loss of potency and stability before other analytical methods.

INTRODUCTION – WHY GENE EXPRESSION?

Potency assays for AAV gene therapy products can measure different points in the process of the AAV cellular transduction (Figure A).

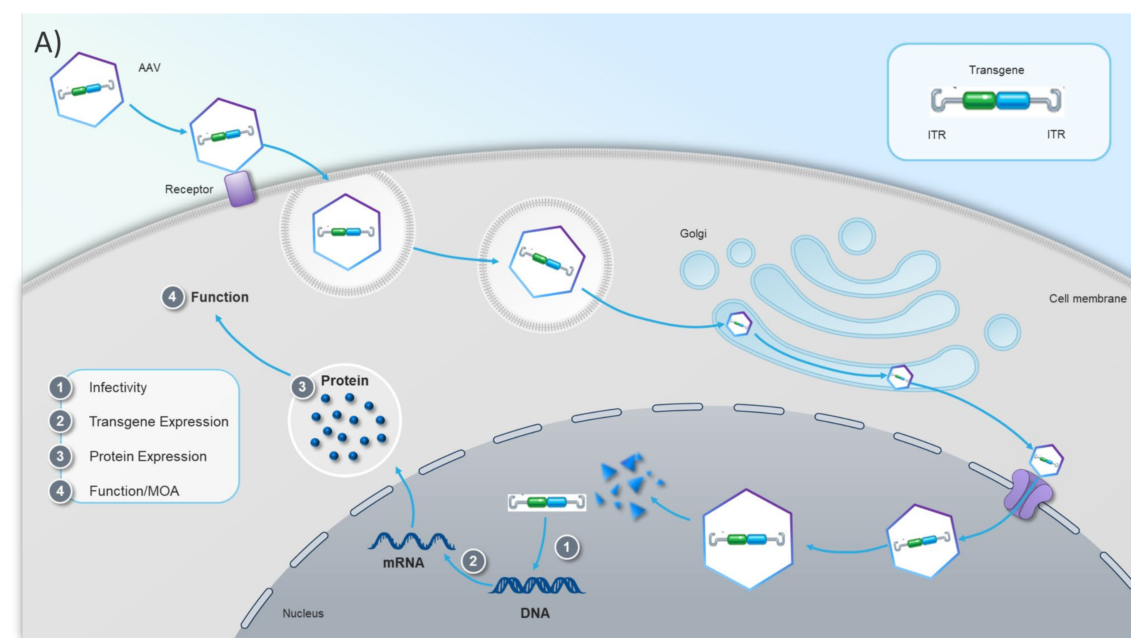

Table B below summarizes the key differences between infectivity, gene expression, and potency.

B)	Infectivity	Gene Expression	Potency
Throughput	1 sample/plate	5 samples/plate	~2 samples/plate
Reported Unit	VG/IU Vector genome/infectious unit	% Relative Gene Expression	% Relative Potency
Platform vs Product	Platform	Platform	Product-Specific

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GENE EXPRESSION PLATFORM

The gene expression platform method has a simple workflow where cells are seeded into a 96-well plate and infected with AAV and an optionally infectivity enhancer. Following a set incubation, mRNA is extracted and a RT-qPCR reaction is performed. Lastly, a delta delta CT analysis is performed to normalize the target gene expression to the housekeeping gene expression. The relative gene expression MOI curve is compared to reference material using a semi-log fit, expressed as a percent. Below is an overview of the experiments performed to establish the gene expression platform.

Cell Line Screening

Several cell lines were screened with different AAV candidates to identify the optimal cell lines for the platform gene expression assay. Figure A below shows an example of two different cell lines screened with the same AAV candidate. Based on the experiments performed during platform optimization, the gene expression platform has several cell lines optimized for different GOI promoters.

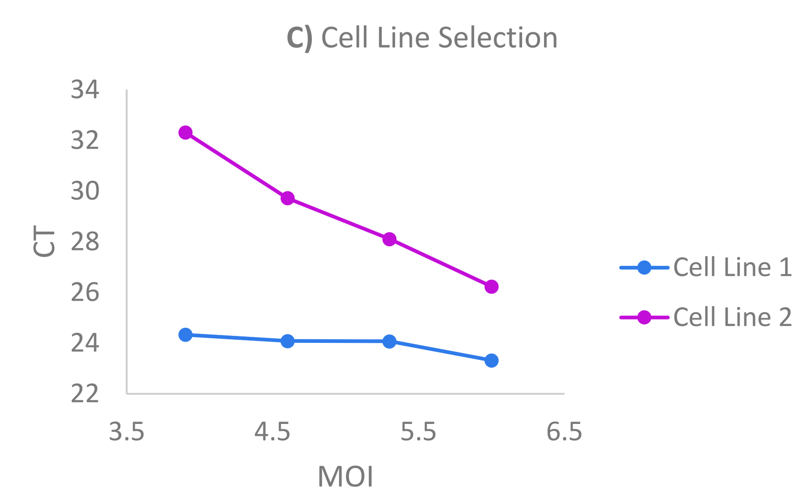


Figure C. Cell Line Selection. Cell line selection experiments were performed with different cell lines to identify a cell line with a dose-dependent response to the AAV candidate. Cell line 2 was selected in this experiment.

Housekeeping Gene

A housekeeping gene must be identified for each cell line used in the platform that has consistent expression. The selected housekeeping gene must also multiplex well with the target gene of interest. Therefore, housekeeping gene selection may be re-evaluated during product development.

mRNA Extraction

There are several mRNA extraction kits available on the market. All kits evaluated had good recovery of mRNA. The carryover of genomic DNA was evaluated in the selection process as well.

DNase

DNase is sometimes necessary to reduce levels of genomic DNA carryover, to ensure that the assay is exclusively measuring mRNA expression. A comparison of different commercially available DNase kits was performed.

RT-qPCR

The gene expression platform assay concludes with an RT-qPCR reaction. Several kits were screened during development. Figure B below shows the results. Several kits have been identified that fit the platform.

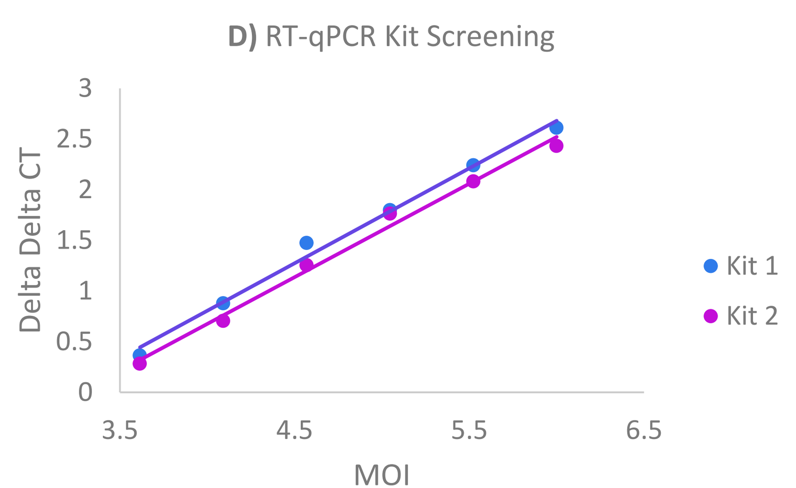
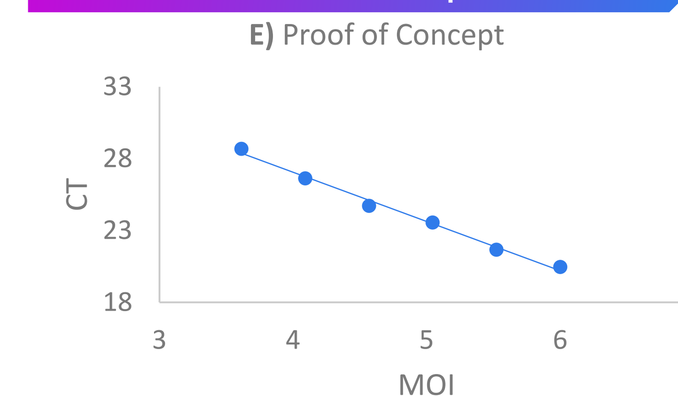


Figure D. RT-qPCR Kit Screening. RT-qPCR kits were screened to optimize the signal in the reaction. Kit 2 was selected

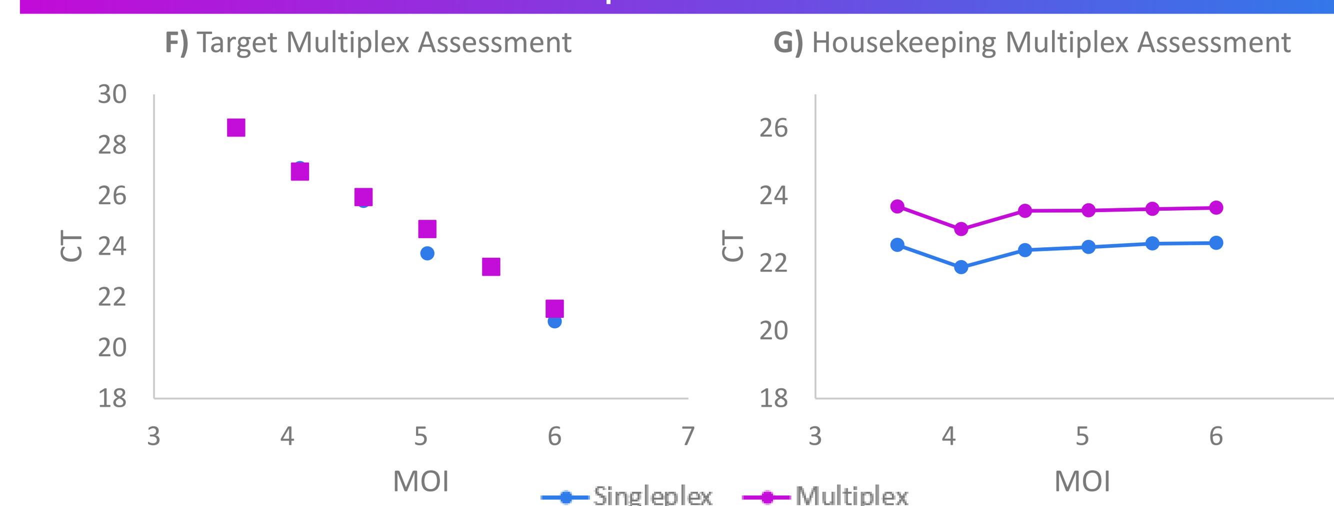
GENE EXPRESSION PRODUCT-SPECIFIC DEVELOPMENT

Once the gene expression platform was established, product specific development could be initiated. The steps below detail the accelerated workflow for gene expression development in the platform. Once these experiments are complete, the assay is ready for process development testing and qualification.

Proof of Concept



Multiplex Assessment



Transduction

MOI Optimization

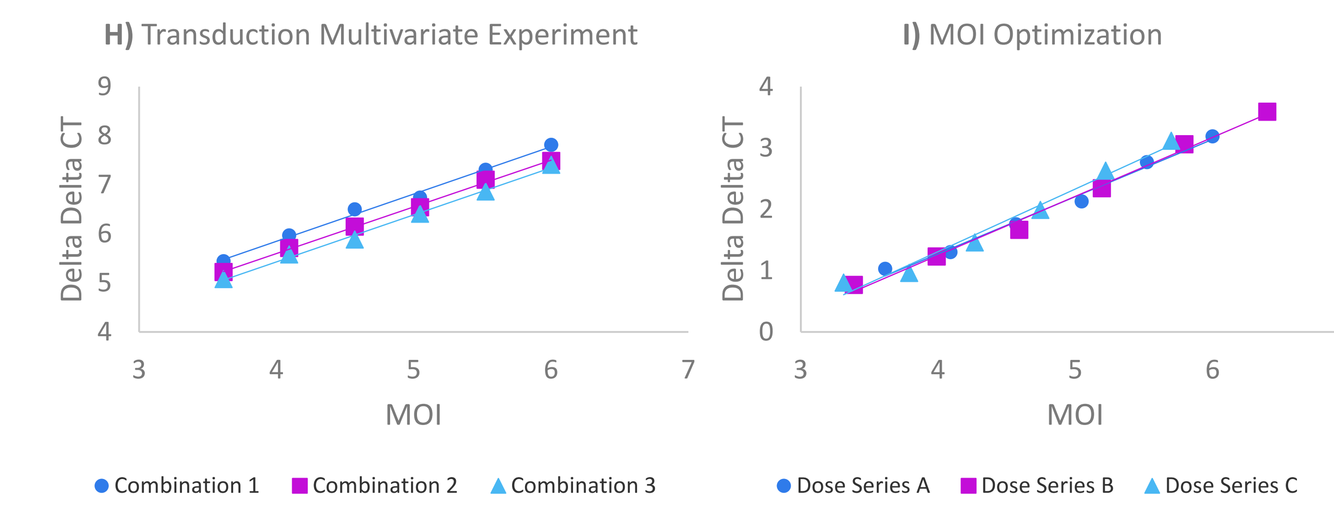
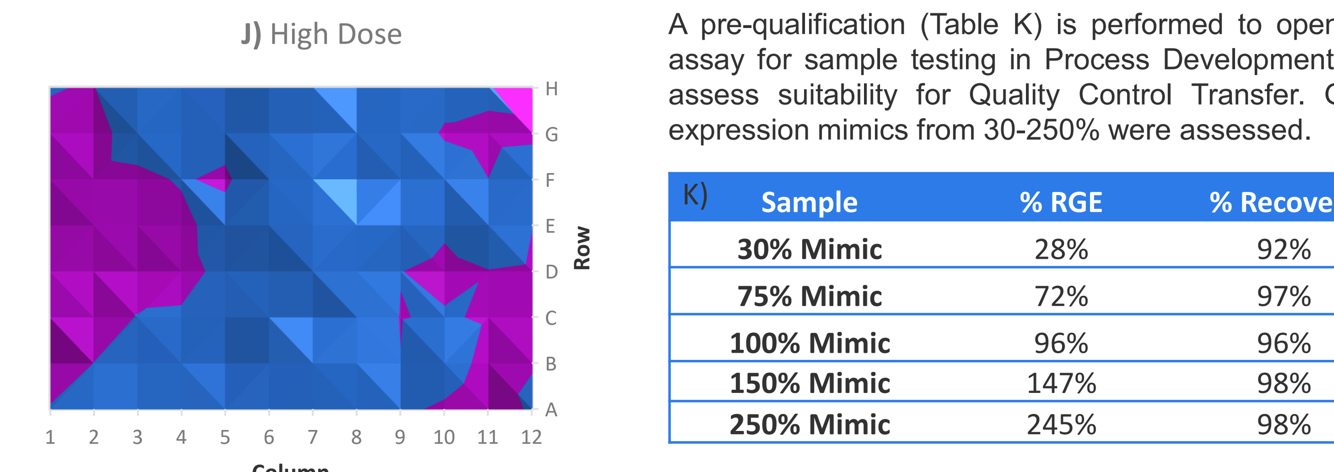


Plate Uniformity

Pre-qualification

A pre-qualification (Table K) is performed to open the assay for sample testing in Process Development and assess suitability for Quality Control Transfer. Gene expression mimics from 30-250% were assessed.



K) Sample	% RGE	% Recovery
30% Mimic	28%	92%
75% Mimic	72%	97%
100% Mimic	96%	96%
150% Mimic	147%	98%
250% Mimic	245%	98%

Figure E. Proof of Concept. The AAV candidate was tested in the platform gene expression method to assess suitability. **Figures F&G. Target and Housekeeping Gene Multiplex Assessment.** The qPCR efficiency between the singleplex and multiplex qPCR reaction was assessed. **Figure H. Transduction Multivariant Assessment.** A comprehensive examination of the transduction parameters (cell seeding density, incubation time, and use of an infectivity enhancer) was assessed. **Figure I. Multiplicity of infection (MOI) Optimization.** Several dose response series were examined to identify the best linear fit. **Figure J. Plate Uniformity for High Dose.** The uniformity of the response for both the high and low MOI was examined to ensure no bias in the sample plate.

STABILITY ASSESSMENT

Potency is a primary stability indicator for AAV gene therapies. Therefore, the gene expression assay must be stability-indicating. The attributes in the Table L were analyzed for stability-assessment by subjecting the AAV to thermal stress at 25°C for 1 month and 40°C for 10 days. Figures I through Q summarize the results.

L) ATTRIBUTE	TEST METHOD
Vector Genome Titer	ddPCR
Capsid Titer	ELISA
Capsid Purity	Capillary Electrophoresis
Capsid Aggregation	Size Exclusion Chromatography
Post-translational Modifications	LCMS
Infectivity	TCID50 Assay
Potency	Gene Expression Assay

Figure M. Vector Genome Titer Stability. Figure N. Capsid Titer Stability. Vector genome titer (I) and capsid titer (J) were measured using ddPCR and ELISA, respectively. Vector genome titer and capsid titer appeared to be stable across different time points at 25°C and 40°C temperatures.
Figure O. Protein Purity Stability. Figure P. VP1 Area Stability. Figure Q. Aggregation Stability. Protein purity (K), VP1 area (L), and Aggregation (M) appeared stable at 25°C. A loss in protein purity and VP1 area, as well as an increase in aggregation, can be seen at 40°C.
Figure R. Infectivity. Figure S. Gene Expression. Infectivity (N) and Gene Expression (O) appeared stable at 25°C. There was a loss of potency seen for both at 40°C, with gene expression showing a loss of potency before infectivity.
Figure T. Post-translational Modifications (PTMs) at 25°C. Figure U. Post-translational Modifications at 40°C. There appeared to be no increase in post-translational modifications (PTMs) (P) at 25°C. At 40°C, several different PTMs were seen to increase in abundance over time (Q).

CONCLUSIONS

Implementing a robust gene expression platform early in product development has many advantages. A short development time for new AAV drug candidates allows for key early testing of product potency. In our analytical toolbox, the gene expression method is able to detect a change in product stability before other stability-indicating methods.

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